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# The molecular and cellular biology of lung cancer: identifying novel therapeutic strategies

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**Introduction:** Lung cancer is the commonest fatal malignancy in the developed world. Survival rates for lung cancer have not changed significantly over the past 30 years.

**Sources of data:** This report is a systematic review of the literature on our current understanding of lung cancer biology. Searches were carried out using PUBMED. 1990–2010.

**Areas of agreement:** A concerted effort to reduce cigarette smoking and nicotine addiction is required. A better understanding of the biology of lung cancer will lead to the identification of earlier diagnostic markers and improved therapy.

**Areas of controversy:** How chronic inflammatory disorders such as COPD and lung fibrosis contribute to lung cancer development is incompletely understood.

**Growing Points:** Developing novel biological agents to target lung cancer. New microarray-based technologies provide new methods for predicting prognosis and response to treatment.

**Areas timely for developing research:** Developing strategies to target lung cancer stem cells may provide a novel approach for treating drug resistant disease.

**Keywords:** lung cancer/smoking/inflammation/genetics/chemotherapy/signal transduction

## Introduction

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Lung cancer is the commonest fatal malignancy in the developed world causing more than 1 million deaths/year. In the UK, lung cancer kills over 37 000 people/year. Current therapies rarely cure the disease, the high relapse rate coupled with delayed diagnosis results in a poor prognosis and the overall survival rate is about 10%. With the increase in cigarette smoking in developing countries, especially India and China, lung cancer will remain a major health problem for the foreseeable future.

About 75–80% of lung cancers are caused by smoking. It is important to reduce cigarette smoking, stop young people from starting and help people stop through smoking cessation programmes and banning smoking in public places. However, about 50% of all new lung cancers are diagnosed in ex-smokers and ~25% of all lung cancer cases in the world occur in never smokers (defined as a lifetime exposure of less than 100 cigarettes). Worldwide it is estimated that 15% of all lung cancers in men and 53% of lung cancers in women are not due to cigarette smoking. Lung cancer in never smokers would rank as the seventh most common cause of cancer deaths worldwide and is more common than deaths from cervical cancer or prostate cancer.<sup>1</sup> Furthermore, chronic inflammatory diseases of the lung such as chronic obstructive pulmonary disease (COPD) and lung fibrosis are significant independent risk factors for developing lung cancer.

In the UK, ~50% of patients present with advanced disease and are not amenable to curative treatment. Increasing public awareness of symptoms and developing biomarkers for early detection may lead to earlier diagnosis. The majority of lung cancers arise from epithelial cells. The two most prevalent histological types of lung cancer are non-small-cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC) causing ~80% and 18% of all lung cancer, respectively. There are three main subtypes of NSCLCs: squamous cell lung carcinoma, adenocarcinoma and large cell lung carcinoma. A better understanding of the molecular and cellular biology of lung cancer, the mechanisms causing lung cancer in never smokers and defining the common molecular pathways by which chronic inflammatory diseases lead to the initiation of lung cancer may identify biomarkers and screening tests, in addition to identifying more effective therapeutic strategies and targets.

## Smoking and lung cancer

Cigarette smoke contains over 60 known carcinogens particularly polycyclic aromatic hydrocarbons (e.g. benzo[a]pyrene) and nitrosamines. Cytochrome P450 enzymes metabolically activate these polycyclic aromatic hydrocarbons and nitrosamines, which can then bind to DNA leading to DNA adducts. Glutathione-S-transferases protect against DNA adduct formation by detoxifying carcinogen intermediates. DNA adducts are mostly repaired; however, chronic DNA adduct formation can lead to mutations in genes such as *p53* that are central to lung cancer initiation.<sup>2</sup>

Tobacco smoke also contains a large amount of free radical oxygen that can cause oxidation of the DNA nucleobase guanine to form

8-oxoguanine resulting in G to T substitutions and gene mutations. 8-Oxoguanine is repaired by 8-oxoguanine DNA *N*-glycosylase 1 (OGG1). Studies suggest that low OGG1 activity increases the risk of lung cancer among smokers. Furthermore, OGG1 Ser326Cys genetic polymorphisms influence the risk of lung cancer, the Cys/Cys genotype is associated with an increased risk of lung cancer compared with the Ser/Ser genotype among heavy smokers.<sup>3</sup>

Genetic studies have identified a nicotine dependence locus on chromosome 15q24-25, which includes the  $\alpha 5$ - $\alpha 3$ - $\beta 4$  nicotinic receptor gene cluster. A polymorphism, which alters an amino acid in the  $\alpha 5$  nicotinic receptor subunit also influences the risk for lung cancer, COPD and vascular disease. In addition, nicotine inhibits apoptosis and can act as a tumour promoter in lung epithelial cells. Varenicline is a partial nicotinic receptor agonist, which reduces nicotine craving. Bupropion inhibits noradrenaline and dopamine reuptake to improve the cessation rates. Genetic profiling of individual smokers may identify the most suitable pharmacologic agent to aid smoking cessation.<sup>4</sup>

## Lung cancer in never smokers

The clinical presentation of lung cancer in never smokers is strikingly different from that of smokers. Adenocarcinoma is the predominant form of lung cancer in never smokers in contrast to smokers who develop all major histological types of lung cancer. In patients less than 40 years old, adenocarcinoma was the commonest form of lung cancer with a disproportionately high ratio of never smokers. In smokers, adenocarcinoma occurs both centrally and in the peripheral airways; however, adenocarcinoma in never smokers is predominantly found in the peripheral airways. Lung cancer in never smokers is more common in women than men. In South East Asia, over 80% of females with lung cancer are never smokers compared with 15% in the USA.<sup>5</sup> Lung cancer patients, who are never smokers, present at an earlier age in South East Asia, compared with the USA and Europe where lung cancer is diagnosed at the same age as smokers. Studies suggest that never smokers present with more advanced stage lung cancer but have improved survival compared with smokers, independent of other prognostic factors.<sup>6</sup> It is unclear whether never smokers respond differently to chemotherapy compared with current and former smokers, as studies are conflicting.

Passive smoking, mainly due to spousal and workplace exposure, is associated with a 10–15% increased risk of developing lung cancer and urine metabolites of tobacco-specific carcinogens are detectable in non-smokers passively exposed to cigarette smoke.<sup>7</sup> Other risk factors

including environmental, hormonal, genetic and viral may account each year for about 3000–4000 deaths in the UK and about 16 000–24 000 deaths in the USA. However, no single factor can explain the development of lung cancer in never smokers.

## Chronic inflammation and lung cancer

Chronic lung inflammation predisposes to lung cancer. There is a 4–5 times greater risk for developing lung cancer among those with COPD independent of age or smoking history.<sup>8</sup> Even among non-smokers, the presence of emphysema on CT scan or a history of COPD is significantly associated with an increased risk of developing lung cancer. In never smokers, carrying the  $\alpha 1$  anti-trypsin deficiency allele increases the risk of lung cancer by 2.2-fold, with a significant increase in adenocarcinoma and squamous cell carcinoma.<sup>9</sup> Furthermore, recent studies have suggested that inhaled corticosteroids may reduce the risk of lung cancer in patients with COPD.<sup>10</sup> In addition, patients with scleroderma who smoke are seven times more likely to develop lung cancer than non-smokers and heavier smokers more likely to develop lung cancer than light smokers. Peripheral lung tumours occur earlier after the onset of scleroderma symptoms than bronchogenic tumours, reflecting the inflammatory origin of these tumours.<sup>11</sup> Idiopathic pulmonary fibrosis and pneumoconiosis associated with silica exposure are associated with an independent increased risk of lung cancer. Evidence suggests that recurrent injury and inflammation result in genetic alterations that predispose to lung cancer, again smoking may play a significant role.

Chronic infections such as hepatitis B or C virus and the Epstein–Barr virus are associated with the development of cancer. The airways of a healthy lung are sterile; however, microbial colonization and associated inflammation develop early in the course of COPD and may persist in ex-smokers with COPD. Failure of the innate immune system in the lung in COPD allows the establishment of chronic infection. Chronic infection further disrupts the innate lung defenses driving a vicious circle of infection and inflammation in COPD. Experiments in mice have shown that infecting the lung with non-typeable *Haemophilus influenza* caused a COPD-like bronchial inflammation that promotes lung cancer development.<sup>12</sup> The co-existence of tuberculosis (TB) and lung cancer is well described. A study in India reported that TB lesions, either active or healing, were found in the lungs of 30–33% of patients with bronchogenic tumours when compared with 7% in the general population.<sup>13</sup> Repeated tissue damage and regeneration as a result of inhalation of environmental toxic pollutants such as

exhaust fumes, and sulphur dioxide as well as microorganisms results in lung injury and generation of reactive oxygen/nitrogen species (ROS/RNS) such as superoxide, hydrogen peroxide and nitric oxide, which then interact with DNA in proliferating epithelium. Although cells respond to DNA damage by activating p53-controlled genes associated with the cell cycle and DNA repair. In chronic inflammation, the rate of ROS/RNS-mediated DNA damage is extensive, increasing the risk of permanent genetic damage and lung cancer.<sup>14</sup>

Chronic persistent inflammation may also cause the usually quiescent bronchoalveolar stem cells (BASCs) to proliferate and repopulate the damaged areas in order to restore the lung architecture. This repeated lung injury and repair driven by chronic inflammation and tissue damage results in increased BASCs proliferation in conjunction with smoking/inflammation-related genetic damage results in cancerous transformation of lung epithelial cells. In a mouse model of K-ras-induced carcinogenesis, BASCs give rise to lung adenocarcinoma. Oncogenic KRAS stimulates the expansion of a cell population that expresses stem cell markers at the region of bronchoalveoli duct junctions, suggesting that these cells are precursors of lung adenocarcinoma. Potentially, BASCs may be a key link between COPD and lung cancer.<sup>15</sup>

Understanding the common processes/molecules that are central to chronic inflammation and cancer may help identify novel therapeutic strategies to prevent and treat lung cancer in addition to identifying biomarkers to target those at high risk of developing lung cancer.

## Proto-oncogene: the epidermal growth factor receptor family and downstream signalling mediators

The epidermal growth factor receptor (EGFR) families are transmembrane tyrosine kinase receptors which induce cell proliferation and enhance resistance to chemotherapy and radiotherapy. The *EGFR* family *EGFR1-4* also named *HER1-4* are 59–81% homologous in the tyrosine kinase domains. HER2 does not bind a specific ligand. HER2 is the preferred heterodimerization partner for other HER family receptors. HER2-containing heterodimers are the most potent with regard to downstream signalling activity. *HER3* lacks kinase activity. Epregeulin (which can be released by infected macrophages) binds to both the EGFR and HER4. HER4 is overexpressed in NSCLC, correlates with lymph node metastasis and occurs in early-stage disease.<sup>16</sup>

The EGFR1 and HER2 represent two of the most targeted oncoproteins in cancer with cetuximab, panitumumab, erlotinib and gefitinib

all demonstrating clinical efficacy in phase III trials for the EGFR, and trastuzumab (Herceptin) and lapatinib are FDA-approved against HER2. *EGFR* mutations and gene overexpression invariably correlate with a good tyrosine kinase inhibitor (TKI) response, whereas the consequence of increased EGFR protein expression is conflicting. EGFR mutations in the tyrosine kinase domain occur in 24% of NSCLCs and are more frequently found in adenocarcinomas arising in never smokers (58% compared with 13% in smokers), conferring approximately a 4-fold higher response rate to gefitinib and/or erlotinib.<sup>17</sup> *HER2* mutations occur in 2% of NSCLCs and appear to occur in the same subpopulation as those with EGFR mutations. In NSCLCs particularly adenocarcinomas, *EGFR* is overexpressed in 70% and *HER2* in 30% of patients but both are rarely expressed in SCLC.<sup>18</sup> In contrast, *HER4* mutations tend to occur in males and smokers.

EGFR TKI as first-line treatment in never smokers causes longer progression-free survival compared with a standard platinum-based chemotherapy.<sup>19</sup> Strong evidence recommends against the use of gefitinib or erlotinib in combination with chemotherapy or as maintenance therapy after chemotherapy and radiation as a first-line treatment. The phase III INTEREST study showed that gefitinib in unselected, pre-treated patients, improved quality of life, reduced toxicity with equivalent overall survival compared with docetaxel chemotherapy. The phase III IPASS study found improved progression-free survival with gefitinib compared with paclitaxel–carboplatin chemotherapy in chemotherapy-naïve, never/light ex-smokers with adenocarcinoma. This and subsequent prospective studies showed that patients with EGFR mutations had longer progression-free survival and higher objective response rates with gefitinib compared with chemotherapy.<sup>20</sup> EGFR mutations are found in histologically normal bronchial epithelial cells next to tumours with EGFR mutations suggesting that EGFR mutation status may be a useful early marker and chemoprevention target for lung cancer.<sup>21</sup>

Approximately 30% of patients with EGFR mutations exhibit primary resistance to EGFR TKI therapy. The mutation T790M in the tyrosine kinase domain of *EGFR* may confer resistance to TKIs and is also reported to be associated with familial NSCLC.<sup>22</sup> MET amplification is detected in 22% of lung cancer specimens that had developed resistance to gefitinib or erlotinib. Met protein expression and phosphorylation is also associated with primary resistance to EGFR TKI therapy in non-small-cell lung cancer patients harbouring EGFR mutations.<sup>23</sup> MET causes gefitinib resistance by driving HER3-dependent activation of phosphatidylinositol-3 kinase (PI-3K). The development of technology to detect EGFR mutations will help patient selection in the future.



The RAS/RAF/MEK/ERK pathway regulates downstream cellular responses to growth factors and growth factor receptors and is upregulated in many cancers. About 10–15% of NSCLCs have activating RAS mutations, particularly adenocarcinoma, of which 20–30% have RAS mutations and 90% of RAS mutations in lung cancers are KRAS mutations, which activate MEK and ERK pathways.<sup>24</sup> KRAS mutations are more frequently found in adenocarcinomas arising in smokers, 21% compared with 4% in never smokers. In addition, EGFR and KRAS mutations appear to be mutually exclusive. RAS mutations are almost never found in SCLC. A number of drugs that target RAS function are in clinical trials. Farnesyl transferase inhibitors which are bioavailable are in phase III clinical trials in lung cancer in combination with cytotoxic drugs. The presence of KRAS mutation is a negative predictor of response to TKIs, and the TRIBUTE trial suggests that treating KRAS-mutated lung cancers with TKIs may be deleterious.<sup>25</sup> Mutations of B-RAF a downstream effector of the RAS pathway occur in 3% of non-small-cell lung cancers. Sorafenib is an orally administered RAF kinase inhibitor. In phase II clinical trials, it has shown no clear benefit in combination with carboplatin/paclitaxel in first-line NSCLC. Activated B-RAF phosphorylates and activates MEK1 and MEK2 which in turn phosphorylates and activates ERK1 and 2. ERK1 and 2 are constitutively activated in a subset of lung cancer cell lines and are therefore therapeutic targets for lung cancer treatment. An oral MEK inhibitor, CI-1040 lacked efficacy in a clinical trial for lung cancer; however, its low toxicity has stimulated further research to find novel compounds with enhanced potency against MEK.<sup>25</sup>

The PI-3K/AKT/PTEN pathway links lipid kinase and tyrosine kinase pathways and is a key regulator of cancer growth, metastasis and treatment failure. PI-3K is constitutively activated in NSCLC and SCLC cell lines. A PI-3K inhibitor LY294002 enhances the sensitivity of lung cancer cell lines to chemotherapeutic agents and radiotherapy and may be useful as a cytotoxic or chemosensitizing agent. Activating mutations of the catalytic subunit of PI-3K occur in 3% of non-small-cell lung cancers. The tumour suppressor gene Phosphatase and Tensin homologue deleted on chromosome 10 (PTEN) is a negative regulator of the downstream effector of PI-3K, AKT. PTEN is rarely mutated in lung cancers but reduced or lost PTEN protein expression is common in lung cancers, resulting in activation of the AKT pathway. Mammalian target of rapamycin (mTOR) is a downstream effector of AKT kinase, and mTOR inhibitors such as sirolimus and its derivatives are now in clinical trials for lung cancer.<sup>26</sup>

Although fusion proteins are rare in the pathogenesis of lung cancer, a gene fusion between echinoderm microtubule-associated protein-like 4 and the anaplastic lymphoma kinase (ALK) gene occurs in ~7% of



NSCLCs, resulting in an activation of a potent ALK fusion protein, which is usually found in never smoker with adenocarcinoma. This ALK fusion protein may play a role in activating RAS and is negatively associated with KRAS or EGFR mutations.<sup>27</sup>

BRCA1 is central to the repair of DNA damage and is an important modulator of the response to chemotherapy. Retrospective and prospective data indicate that low BRCA1 mRNA levels predict better response and survival when patients are treated with cisplatin, non-taxane combinations.<sup>28</sup>

BCL2 protects cells against apoptosis. BCL2 is overexpressed in 10–35% of NSCLC and 75–95% of SCLC. Inhibitors of BCL2, BCL-X<sub>L</sub> and BCL-w have anti-lung cancer activity in the mouse xenograft models. However, surprisingly BCL2 expression and BAX, a BCL2-related protein, correlate with a good prognosis in SCLC, and clinical trials of anti-BCL2 agents in combination with the standard chemotherapy regimens did not improve the clinical outcome.<sup>29</sup>

## Cell-cycle regulation

*p53* has been described as the guardian of the genome and is one of the earliest mutations seen during lung carcinogenesis, leading to genetic instability. *p53* is a transcription factor, located on chromosome 17p13.1. Cytotoxic injury from DNA damaging agents or hypoxia causes stabilization of the *p53* protein leading to the expression of cell-cycle arrest genes, allowing either DNA repair or the initiation of apoptosis. Ninety percent of SCLCs and 50% of NSCLCs have inactivating mutations of the *p53* gene. TP53 mutations are more commonly found in smokers compared with never smokers who develop adenocarcinoma, 71% compared with 48%, respectively. TP53 mutation patterns are different in lung cancers arising in smokers and never smokers. G:C to T:A and A:T to G:C at CpG sites are more common in smokers, 45% compared with 10% in never smokers. In contrast, G:C to T:A at non-GpC sites is more common in never smokers, 29% compared with 4% in smokers.<sup>30</sup> Re-expressing a wild-type *p53* in lung cancer cells induces apoptosis. Clinical trials of *p53* gene replacement therapy have demonstrated the feasibility and safety of gene therapy and showed evidence of anti-tumour activity in a number of cancers including lung cancer.<sup>31</sup>

The pRb(retinoblastoma)/p16 pathway plays an essential role in tumour suppression in lung epithelium. In the hypophosphorylated state, pRb is active and carries out its role as tumour suppressor by inhibiting cell-cycle progression. Hyperphosphorylation by the cyclin D1/CDK4 inactivates pRb, during the M-to-G1 transition. Loss or

mutation of *Rb* is found in 15–30% of NSCLCs and over 90% of SCLC.<sup>32</sup> p16<sup>INK4α</sup> prevents CDK4 from phosphorylating RB. The loss of p16<sup>INK4α</sup> function results in hyperphosphorylation and inactivation of pRb. p16<sup>INK4α</sup> is inactivated by mutations, homozygous deletions and promoter hypermethylation in 70% of NSCLCs. Aberrant p16<sup>INK4α</sup> promoter DNA methylation can be found in sputum or exfoliated lung cells very frequently in lung cancer patients and among disease-free smokers correlates only with very heavy smoking (>50 pack years) and therefore might be a useful biomarker.<sup>33</sup> Cyclin D1 is overexpressed in over 40% of NSCLCs. Overexpression of cyclin D1 in histologically, normal bronchial epithelium in non-small-cell lung cancer patients is associated with cigarette smoking and shorter survival. Thus, cyclin D1 may be a useful biomarker to identify high-risk subjects.<sup>34</sup>

## Neuropeptide growth factors in small-cell lung cancer

SCLCs secrete many hormones and vasoactive peptides such as gastrin-releasing peptide (GRP) and vasopressin. Patients present with syndromes related to ectopic hormone secretion such as inappropriate anti-diuretic hormone secretion and high levels of vasopressin in serum predicts a poorer prognosis in patients with SCLC. Multiple neuropeptides act as growth factors and drive the unrestrained proliferation of SCLC cells. The expression of these neuropeptide receptors increases as cells become resistant to chemotherapy. 2A11 a monoclonal antibody that binds GRP preventing receptor interaction has been shown to inhibit the growth of SCLC *in vitro* and in xenografts in nude mice. Analogues of substance P, such as [Arg<sup>6</sup>, D-Trp<sup>7,9</sup>, N<sup>me</sup>Phe<sup>8</sup>]-substance P (6–11) (SP-G), bind to multiple neuropeptide receptors on SCLC cells and block the mitogenic effects of neuropeptides and inhibit the growth of SCLC cells *in vitro* and *in vivo*. SP-G has been shown to sensitize SCLC cells to chemotherapy and may have an additional benefit in resistant disease. SP-G has been taken into phase I clinical studies for SCLC where therapeutic plasma levels were achieved with no dose limiting toxicity.<sup>35</sup>

## Cell adhesion-mediated drug resistance

Evidence suggests that the development of chemoresistance in SCLC results from interactions between SCLC cells and extracellular matrix (ECM) in the local tumour microenvironment. Fibronectin, collagen IV and laminin are found in the stroma around the areas of tumour

infiltration. Adhesion of SCLC cells to these ECM proteins stimulates  $\beta 1$  integrin-mediated PI3-kinase activation, which prevents chemotherapy (and radiation)-induced cell-cycle arrest and apoptosis despite persistent DNA damage. ECM promotes SCLC cell survival during chemotherapy and radiotherapy. Thus, blocking  $\beta 1$  integrin or its downstream signals may be a novel therapeutic strategy to improve responses to chemotherapy and radiotherapy. Clinical studies have shown that high levels of ECM proteins around SCLC or high expression of  $\beta 1$  integrins on SCLC cells predict a poor prognosis with significantly shorter survival. This may potentially be used as a marker to select SCLC patients for more intensive treatment.<sup>36</sup>

## Vascular endothelial growth factor

Tumour growth beyond 2 mm<sup>3</sup> requires angiogenesis and the amount of angiogenesis, as measured by microvessel density, significantly correlates with metastasis and poor survival in lung cancer. Lung cancers produce high levels of VEGF. Bevacizumab is a monoclonal antibody that neutralizes all VEGF isoforms and has been tested in clinical trials. The combination of bevacizumab with paclitaxel and carboplatin provided a significant survival advantage in advanced adenocarcinoma.<sup>37</sup> Serious life threatening side effects due to haemorrhage have been observed. ZD6474 is a dual inhibitor of the VEGF receptor, and EGFR and a phase 2 clinical trial using the combination of ZD6474 and docetaxel as a second-line therapy showed an improved progression-free survival in patients with advanced disease.<sup>38</sup>

## Immune responses in lung cancer

Immune responses play an important role in the development of cancers. Patients who are immunosuppressed are at greater risk of developing cancer. Spontaneous tumour regression suggests that tumours can be rejected by immunological responses. Lung cancers escape immune surveillance by expressing FAS ligand, which causes apoptosis of activated T-cells. In lung cancer cells, there is also downregulation of the major histocompatibility complex class 1 molecule, preventing the presentation of endogenous antigen peptides to cytotoxic T-cells. Vaccination for lung cancer is difficult because of the poor antigenic properties of lung cancer.

## Lung cancer stem cells

Cancer stem cells divide asymmetrically giving rise to another cancer stem cell, and a cancer progenitor cell that contributes to the tumour cell population. It is proposed that cancer stem cells may be responsible for resistance to chemotherapy and radiotherapy because of their low proliferation rate and the cell surface expression of drug transporters. Increased numbers of stem cells confers a worse prognosis in haematological malignancies, breast and CNS tumours. However, in lung cancer, there is as yet no definitive evidence for cancer stem cells. Further evidence is required to prove their existence and define the phenotype of lung cancer stem cells, which may prove to be important therapeutic targets to treat and eradicate residual disease.

## Genetic factors in lung cancer

Genetic factors may influence a person's susceptibility to lung cancer. A family history of lung cancer, after controlling for cigarette smoke, is associated with a 2-fold increased risk of lung cancer with evidence of risk related to early age of diagnosis and number of relatives affected.<sup>39</sup> A large-scale linkage analysis suggested that 6q23-25 is a major autosomal susceptibility locus for inherited lung cancer. This region contains many genes potentially involved in the development of lung cancer.<sup>40</sup> Common polymorphisms in this region could be used to identify people who may be suitable for smoking cessation and screening programmes.

Lung cancers show genetic instability which results in losses or gains of whole or large portions of chromosomes. The exact causes of chromosomal instability are uncertain but may be due to mutations in mitotic checkpoint genes. Another form of chromosomal instability is microsatellite instability that results in DNA sequence changes. Altered DNA sequences either microsatellite instability or loss of heterozygosity can be found in 43% of blood samples from patients with stage 1 lung cancer. No alterations were found in control cases suggesting that this may be a useful tool for early diagnosis and screening.<sup>41</sup>

Epigenetic changes are common in lung cancer. Hypermethylation of cytosine in clusters of CpG dinucleotides in the DNA promoter sequence of protein-coding genes can lead to the loss of gene expression. In lung cancer, over 80 genes are hypermethylated including tumour suppressor genes, e.g. *p16<sup>INK4α</sup>*. Detecting methylated DNA in sputum or blood may be a useful biomarker for early detection of lung cancer. Studies have shown that detecting three or more

methyated genes from a panel of six selected genes correlated with a 6.5-fold increase in lung cancer with a sensitivity and specificity of 64%.<sup>42</sup> In addition, another epigenetic change that inhibits gene expression is histone deacetylation. Promotor methylation and histone deacetylation are reversible processes; thus, pharmacological inhibition is a potential novel therapeutic strategy that may reverse gene silencing and thus be efficacious in lung cancer.

Repetitive TTAGGG telomere sequences localized at the end of mammalian chromosomes protect chromosomes from degradation. Telomeres shorten after each round of cell division, limiting the life span of the cell. Telomerase maintains telomeres by elongating telomeric DNA by reverse transcription. Upregulation of telomerase is thought to contribute to early immortalization of cells, and telomerase is upregulated in 80% of NSCLCs and nearly 100% of SCLCs. In NSCLCs, high telomerase activity correlates with high proliferation rates and advanced stage. GRN163L (imetelstat), a telomerase antagonist inhibits growth *in vitro* and *in vivo* of lung cancer cells and may block cancer stem cells, is about to enter phase II clinical trials in lung cancer.<sup>43</sup>

Lung cancer classification based on microarray gene expression profiles match conventional histological classification and correlate with patient survival. This may help target patients at high risk of recurrence who may benefit from adjuvant chemotherapy. Gene expression profiles from human airway epithelial cells identify ~100 genes differentially expressed between current and never smokers. Expression of potential tumour suppressor genes and oncogenes persist after smoking cessation, which may account for the fact that 50% of all lung cancer cases occur in ex-smokers. Models that integrate clinical data and multiple gene expression profiles are significantly better at predicting recurrence than those using clinical data only. Combining cytopathology and gene expression profiles improves the diagnostic sensitivity of lung cancer to 95% with 95% negative predictive value.<sup>44</sup> Thus, microarray is an exciting new tool to classify lung cancers and predict prognosis and response to treatments. Next-generation sequencing technologies have recently sequenced two lung cancer genomes revealing the mutational processes, repair pathways and gene networks associated with lung cancer development. First, the genome of an SCLC cell line derived from a bone marrow<sup>45</sup> and second comparing a primary lung adenocarcinoma with adjacent normal tissue.<sup>46</sup> Both showed mutation signatures characteristic of tobacco carcinogens. A total of 22 910–50 000 point mutations were identified with an estimated genome-wide somatic mutation rate of ~17/Mb, some of which may be partially redundant. Further sequencing will identify recurrent lung cancer initiating mutations.

## Summary

Further research is required to identify the markers for early detection and validate screening methods for patients at high risk of lung cancer. In addition, identifying genetic and biological factors that predict response to conventional and novel biological therapies and stratify patients into high-risk groups requiring further intensive therapy is required. Understanding the molecular and biological mechanisms that drive the initiation and progression of lung cancer have already identified and will in the future identify further targets. Drug resistance limits the current therapeutic approaches. Characterization of the mechanisms that lead to the acquisition of drug resistance, in addition to the evaluation of lung cancer stem cells, will help develop strategies to combat drug resistance. It is possible that with further validation of predictive biomarkers and tumour genetic signatures, it will be possible to specifically tailor treatment decisions in individual lung cancer patients. Ultimately, greater understanding of the molecular events that drive lung cancer and genetic mutations that determine sensitivity to conventional and targeted therapies may allow lung cancers to become a chronic disease with relapses and remissions with which patients can have prolonged survival.

## References

- 1 Parkin DM, Bray F, Ferlay J *et al.* Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
- 2 Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* 2003;3:733–44.
- 3 Chang CH, Hsiao CF, Chang GC *et al.* Interactive effect of cigarette smoking with human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) polymorphisms on the risk of lung cancer: a case–control study in Taiwan. *Am J Epidemiol* 2009;170:695–702.
- 4 Bierut LJ. Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. *Trends in Pharmacol Sci* 2010;31:46–51.
- 5 Wakelee HA, Chang ET, Gomez SL *et al.* Lung cancer incidence in never smokers. *J Clin Oncol* 2007;25:472–8.
- 6 Tammemagi CM, Neslund-Dudas C, Simoff M *et al.* Smoking and lung cancer survival: the role of comorbidity and treatment. *Chest* 2004;125:27–37.
- 7 Samet JM, Avila-Tong E, Boffetta P *et al.* Lung cancer in never smokers: clinical epidemiology and environmental risk factors. *Clin Can Res* 2009;15:5626–45.
- 8 Lee G, Walser TC, Dubinett SM. Chronic inflammation, chronic obstructive pulmonary disease, and lung cancer. *Curr Opin Pulm Med* 2009;15:303–7.
- 9 Yang P, Sun Z, Krowka MJ *et al.* Alpha1-antitrypsin deficiency carriers, tobacco smoke, chronic obstructive pulmonary disease, and lung cancer risk. *Arch Intern Med* 2008;168:1097–103.
- 10 Kiri VA, Fabbri LM, Davis KJ *et al.* Inhaled corticosteroids and risk of lung cancer among COPD patients who quit smoking. *Respir Med* 2009;103:85–90.
- 11 Pontifex EK, Catherine L, Hill CL *et al.* Risk factors for lung cancer in patients with scleroderma: a nested case–control study. *Ann Rheum Dis* 2007;66:551–3.

- 12 Moghaddam SJ, Li H, Cho SN *et al.* Promotion of lung carcinogenesis by chronic obstructive pulmonary disease-like airway inflammation in a K-ras-induced mouse model. *Am J Respir Cell Mol Biol* 2009;40:443–53.
- 13 Nalbandian A, Yan BS, Pichugin A *et al.* Lung carcinogenesis induced by chronic tuberculosis infection: the experimental model and genetic control. *Oncogene* 2009;28:1928–38.
- 14 Cook JA, Gius D, Wink DA *et al.* Oxidative stress, redox, and the tumor microenvironment. *Semin Radiat Oncol* 2004;14:259–66.
- 15 Houghton AM, Mouded M, Shapiro SD. Common origins of lung cancer and COPD. *Nat Med* 2008;14:1023–4.
- 16 Sato M, Shames DS, Gazdar AF *et al.* A translational view of the molecular pathogenesis of lung cancer. *J Thorac Oncol* 2007;4:327–43.
- 17 Pao W, Miller V, Zakowski M *et al.* EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;101:13306–11.
- 18 Sekido Y, Fong KM, Minna JD. Molecular genetics of lung cancer. *Annu Rev Med* 2003;54:73–87.
- 19 Mok T, Wu YL. A small step towards personalised medicine for non-small cell lung cancer. *Discov Med* 2009;8:227–31.
- 20 Campbell L, Blackhall F, Thatcher N. Gefitinib for the treatment of non-small-cell lung cancer. *Expert Opin Pharmacother* 2010;11:1343–57.
- 21 Tang X, Varella-Garcia M, Xavier AC *et al.* Epidermal growth factor receptor abnormalities in the pathogenesis and progression of lung adenocarcinomas. *Cancer Prev Res* 2008;1:192–200.
- 22 Bell DW, Gore I, Okimoto RA *et al.* Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* 2005;37:1315–6.
- 23 Benedettini E, Sholl LM, Peyton M *et al.* Met activation in non-small cell lung cancer is associated with de novo resistance to EGFR inhibitors and the development of brain metastasis. *Am J Pathol* 2010;177:415–23.
- 24 Aviel-Ronen S, Blackhall FH, Sheperd FA *et al.* K-ras mutations in non-small cell lung carcinoma: a review. *Clinical Lung Cancer* 2006;8:30–8.
- 25 Wong KK. Recent developments in anti-cancer agents for the Ras/MEK/ERK pathway. *Recent Pat Anticancer Drug Discov* 2009;1:28–35.
- 26 Memmott RM, Dennis RA. The role of AKT/mTOR pathway in tobacco carcinogen-induced lung tumorigenesis. *Clinical Can Res* 2010;16:4–10.
- 27 Soda M, Choi YL, Enomoto M *et al.* Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–6.
- 28 Rosell R, Vergnenegre A, Liu B *et al.* Biomarkers in lung oncology. *Expert Opin Pharmacother* 2010;11:1683–93.
- 29 Rudin CM, Salgia R, Wang X *et al.* Randomised phase II study of carboplatin and etoposide with or without the bcl2-antisense oligonucleotide oblimersen for extensive stage small cell lung cancer. *J Clin Oncol* 2008;26:870–6.
- 30 Subramanian J, Govindan R. Molecular genetics of lung cancer in people who have never smoked. *Lancet Oncol* 2008;7:676–82.
- 31 Roth JA. Gene therapy in thoracic oncology. *Ann Thorac Surg* 2008;85:1837–8.
- 32 Shimizu E, Coxon A, Otterson GA *et al.* RB protein status and clinical correlation from 171 cell lines representing lung cancer, extrapulmonary small cell carcinoma, and mesothelioma. *Oncogene* 1994;9:2441–8.
- 33 Georgiou E, Valeri R, Tzimagiorgis G *et al.* Aberrant p16 promoter methylation among Greek lung cancer patients and smokers: correlating with smoking. *Eur J Cancer Prev* 2007;16:396–402.
- 34 Ratschiller D, Heighway J, Gugger M *et al.* Overexpression in bronchial epithelia of patients with lung cancer is associated with smoking and predicts survival. *J Clin Oncol* 2003;21:2085–93.
- 35 Hodgkinson PS, Mackinnon A, Sethi T. Targeting growth factors in lung cancer. *Chest* 2008;133:1209–16 [Review].
- 36 Hodgkinson PS, Mackinnon AC, Sethi T. Extracellular matrix regulation of drug resistance in small-cell lung cancer. *Int J Radiat Biol* 2007;83:733–41.



- 37 Ramalingam SS, Dahlberg SE, Langer CJ *et al.* Outcomes for elderly, advanced-stage non small-cell lung cancer patients treated with bevacizumab in combination with carboplatin and paclitaxel: analysis of Eastern Cooperative Oncology Group Trial 4599. *J Clin Oncol* 2008;**26**:60–5.
- 38 Natale RB. Dual targeting of the vascular endothelial growth factor receptor and epidermal growth factor receptor with Vandetanib in patients with advanced or metastatic non-small cell lung cancer. *J Thoracic Oncol* 2008;**6**:28–30.
- 39 Matakidou A, Eisen T, Houlston RS. Systematic review of the relationship between family history and lung cancer risk. *Brit J Cancer* 2005;**93**:825–33.
- 40 Bailey-Wilson JE, Amos CI, Pinney SM *et al.* A major lung cancer susceptibility locus maps to chromosome 6q23-25. *Am J Hum Genet* 2004;**75**:460–74.
- 41 Sozzi G, Musso K, Ratcliffe C *et al.* Detection of microsatellite alterations in plasma DNA of non-small cell lung cancer patients: a prospect for early diagnosis. *Clin Cancer Res* 1999;**5**:2689–92.
- 42 Belinsky SA, Liechty KC, Gentry FD *et al.* Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort. *Cancer Res* 2006;**66**:3338–44.
- 43 Röth A, Harley CB, Baerlocher GM. Imetelstat (GRN163L)—telomerase-based cancer therapy. *Recent Results Cancer Res* 2010;**184**:221–34 [Review].
- 44 Beane J, Sebastiani P, Whitfield TH *et al.* A prediction model for lung cancer diagnosis that integrates genomic and clinical features. *Cancer Prev Res (Phila Pa)* 2008;**1**:56–64.
- 45 Pleasance ED, Stephens PJ, O'Meara S *et al.* A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature* 2010;**463**:184–90.
- 46 Lee W, Jiang Z, Liu J *et al.* The mutation spectrum revealed by paired genome sequences from a lung cancer patient. *Nature* 2010;**465**:473–7.